excreted in high concentrations, and as much as 30% of the dose may be detected in bile (Levine & Clark, 1955; Schanker & Solomon, 1963). Tetraethylammonium, hexamethonium and decamethonium, however, are present only in trace amounts in bile (Levine, 1960; Schanker, 1962; Luthi & Waser, 1965). The results reported here were concerned with the biliary excretion of hydroxyphenyltrimethylammonium; this compound has been shown to be the principal metabolite of neostigmine (Roberts, Thomas & Wilson, 1965). Wistar rats were anaesthetized with urethane and the common bile duct was cannulated through a mid-line abdominal incision. Trimethyl-^{14}C-(*m*-hydroxyphenyl)-trimethylammonium iodide (specific activity 10·2 μc/μM; 2 μM/kg) was injected intravenously over a one-minute period, and hepatic bile was collected at hourly intervals for 4 hr.

The amount of radioactivity detected in bile represented less than 3% of the dose. Thus the elimination of hydroxyphenyltrimethylammonium in bile is quantitatively similar to the excretion of neostigmine (Calvey, 1966).

Four main peaks of radioactivity were resolved by paper chromatography of bile in a mixture of *n*-butanol, ethanol, acetic acid and water (Somani, Roberts, Thomas & Wilson, unpublished). Two of these peaks had similar R_f values to concurrently run authentic standards of hydroxyphenyltrimethylammonium and hydroxyphenyldimethylamine. The two remaining peaks were both eliminated by previous incubation with β-glucuronidase, and were tentatively identified as the glucuronides of these two compounds. These two peaks accounted for most of the radioactivity in bile.

On the basis of these results it is suggested that hydroxyphenyltrimethylammonium is mainly excreted in bile as the glucuronide of the parent drug and the glucuronide of its demethylated metabolite. Similar metabolic pathways may well be involved in the biliary excretion of neostigmine.

REFERENCES

CALVEY, T. N. (1966). The biliary excretion of neostigmine in the rat. *Br. J. Pharmac. Chemother.*, **28**, 348–359.

LEVINE, R. R. (1960). The physiological disposition of hexamethonium and related compounds. *J. Pharmac. exp. Ther.*, **129**, 296–304.

LEVINE, R. M. & CLARK, B. B. (1955). The biotransformation, excretion, and distribution of the anticholinergic quaternary ammonium compared with benzomethamine (N-diethylaminoethyl-N-methylbenzilamide methobromide (MC 3199) and its tertiary amine analogue (MC 3137) and related compounds in animals. *J. Pharmac. exp. Ther.*, **114**, 63–77.

LÜTHI, U. & WASER, P. G. (1965). Verteilung und metabolismus von ^{14}C-decamethonium in Katzen. *Archs int. Pharmacodyn. Thér.*, **156**, 319–347.

ROBERTS, J. B., THOMAS, B. H. & WILSON, A. (1965). Metabolism of ^{14}C-neostigmine in the rat. *Br. J. Pharmac. Chemother.*, **25**, 763–770.

SCHANKER, L. S. (1962). Concentrative transfer of an organic cation from blood into bile. *Biochem. Pharmac.*, **11**, 253–254.

SCHANKER, L. S. & SOLOMON, H. M. (1963). Active transport of quaternary ammonium compounds into bile. *Am. J. Physiol.*, **204**, 829–832.

A possible explanation for the varied effects of sulphonic acid diesters on spermatogenesis

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The different antifertility effects in male rodents produced by simple members of a series of methane sulphonic esters (busulphan (Myleran) homologues, n=1–9) is

remarkable (Jackson, 1966). Differing abilities of these diesters to elicit haematological changes or cause regression of transplanted tumours in rats have been attributed to physico-chemical influences, such as solubility (Hudson, Timmis & Marshall, 1958) and chemical reactivity (Timmis, 1958). Antifertility effects after single doses of the simplest esters methylene dimethanesulphonate (MDS, n=1), ethylene dimethanesulphonate (EDS, n=2), propylene dimethanesulphonate (PDS, n=3) and busulphan (n=4) indicates that these compounds are affecting cells in the same environment but at different stages of development, and it is hard to correlate differences in stability, reactivity or solubility with these effects.

$$CH_3SO_2.(CH_2)_n.OSO_2CH_3 \qquad (I)$$

Histological examination of damage produced to rat testis support the conclusions from fertility studies on the locus of action of these compounds on different spermatogenic cells in the testis tubules. Apart from ethylene dimethanesulphonate the main feature is inhibition of spermatogonial development for 4–5 weeks (Jackson, 1964; 1966) although some spermatocyte damage occurs, minimal with busulphan, Methylene dimethanesulphonate also sterilizes epididymal sperm (Fox & Jackson. 1965). Ethylene dimethanesulphonate, on the other hand, will induce aspermia in rats from the second or third week from treatment due to a progressive destructive effect upon first spermatozoa, then spermatids and later spermatocytes. Effects on spermatogonia are minimal.

Chemical and metabolic studies with ethylene dimethanesulphonate suggest that although the compound has low inherent reactivity towards biological sites (in contrast to busulphan), it is capable of producing a highly reactive species once alkylation of a biological site has taken place. For propylene dimethanesulphonate similar influences are envisaged, although in this case the product of alkylation would be of lesser reactive potential because of the three carbon chain and a weaker influence from the alkylated nucleophile. An effective pharmacological action from the latter would require special environmental circumstances, perhaps leading to greater selectivity. In myleran and its higher homologues both alkylating groups have comparable reactive potential and the residual ester group will be now uninfluenced by the nature of an alkylated site. Methylene dimethanesulphonate is unstable in solution, being rapidly hydrolysed to formaldehyde, but metabolic studies using ^{35}S labelled material indicate rapid distribution into rodent tissues after injection. Chemical studies with derivatives of the readily alkylated compound cysteine have not indicated any differences in reaction between formaldehyde and methylene dimethanesulphonate. Methylene dimethanesulphonate may reach target sites and alkylate or deliver formaldehyde locally. However, fertility studies with other chemicals which produce formaldehyde, such as methylene diacetate, so far show little predisposition to damage the spermatogenic epithelium.

It is suggested that ethylene dimethanesulphonate and propylene dimethanesulphonate may be regarded as compounds with latent activity and their antispermatogenic effects, in contrast to busulphan and its higher homologues, could be influenced by this factor.

REFERENCES

FOX, B. W. & JACKSON, H. (1965). In vivo effects of methylene dimethane sulphonate on proliferating cell systems. *Br. J. Pharmac. Chemother.*, **24**, 24–28.

HUDSON, R. F., TIMMIS, G. M. & MARSHALL, R. D. (1958). A physico-chemical investigation into the biological action of Myleran and related sulphonic esters. *Biochem. Pharmac.*, **1**, 48–59.

JACKSON, H. (1964). Effects of alkylating agents on fertility. *Br. med. Bull.*, **20**, 107–114.

JACKSON, H. (1966). In *Antifertility Compounds in the Male and Female.* Springfield: Charles C. Thomas.

TIMMIS, G. M. (1958). Comparative clinical and biological effects of alkylating agents. Part I, Chemistry of alkylating agents. Discussion. *Ann. N.Y. Acad. Sci.*, **68**, 721–730.

Chemosterilant action of trimethylphosphate in rodents

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Trimethylphosphate, the simplest tri-alkyl ester of phosphoric acid, produces marked antifertility effects in experimental male rodents (Jackson & Jones, 1968). The predominant effect is the "functional" sterilizing action involving spermatids from which intact, motile but incompetent sperm continue to be produced. Relatively high doses are required in the mouse (5×1 g/kg orally), whereas it is effective in the rat at one tenth of this level.

Trimethylphosphate is remarkable in that it possesses no anticholinesterase activity, is freely soluble and stable in water, is effective orally and has a high level of tolerance. Whereas 500 mg/kg orally render male rats sterile for the ensuing 3 weeks, five times this amount, although tolerable, completely disorganizes spermatogenesis without damaging tubular architecture. Such treated rats remain infertile for 20–25 weeks, apparently retaining sexual activity, though a proportion appear to be more permanently sterilized. Rats treated weekly at 5×100 mg/kg orally for over one year have remained sterile but recover fertility 3–5 weeks from terminating treatment. "Side-effects" so far observed are a sedative action and, towards one year of treatment, hind leg paresis, although five times this dose rate caused progressive loss in weight.

Using ^{32}P-trimethylphosphate the sole phosphorus-containing metabolite is dimethylphosphate (Jackson & Jones, 1968), which has no antifertility activity. With ^{14}C-trimethylphosphate, *S*-methyl cysteine was identified as a urinary metabolite, indicating that trimethylphosphate is involved, at least in its detoxification process, as an alkylating agent.

The antifertility action of trimethylphosphate is probably related to methyl alkylation. This would bring it into line with the methyl ester of methanesulphonic acid which also produces the "functional" type of sterility in rats and mice (Jackson, 1964). Like methyl methanesulphonate (Partington & Bateman, 1964), trimethylphosphate in sub-sterilizing doses induces so-called dominant lethal mutations.

Preliminary structure/activity studies have shown that tri-ethyl- and tri-*iso*-propyl-phosphates do not affect the fertility of male mice (5×1 g/kg orally). Both these esters together with tri-*n*-propyl- and tri-*n*-butyl-phosphates still have the capacity to alkylate, and like trimethylphosphate, the only metabolites in the rat were the di-alkylphosphates and corresponding *S*-alkyl cysteines. Whereas all these substances interact with cysteine *in vitro*, only trimethylphosphate reacts readily with glutathione. This might be pertinent to its biological activity.

REFERENCES

JACKSON, H. (1964). Effect of alkylating agents on fertility. *Br. med. Bull.*, **20**, 107–114.

JACKSON, H. & JONES, A. R. (1968). Antifertility action and metabolism of trimethylphosphate in rodents. *Nature, Lond.*, **220**, 591.

PARTINGTON, M. & BATEMAN, A. J. (1964). Dominant lethal mutations induced in male mice by methyl methanesulphonate. *Heredity*, **19**, 191–200.